

LEG EXTENSION IN *LIMULUS*

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The ancestors of arthropods were probably worm-like animals with hydrostatic skeletons. With the evolution of a rigid exoskeleton the hydraulic system lost importance as a means of support and leg extension and has apparently disappeared in most adult arthropod forms. Nevertheless hydraulic systems seem to persist in a few groups; such animals typically show relatively large membranous areas where the integument is not stiff, and lack extensor muscles in one or more of their leg joints. Spiders, various myriapods, scorpions, and *Limulus* show both of these characteristics. These characteristics alone, however, are not sufficient evidence for the presence of a hydraulic system. Only in spiders is the existence of such a system well substantiated (Parry and Brown, 1959a, 1959b). In centipedes, millipedes, and scorpions the evidence for the presence of hydraulic leg extension is equivocal (Manton, 1958a). We have no evidence other than the above characteristics that a hydraulic system exists in *Limulus*. The problem is especially interesting in *Limulus* since the horseshoe crab is considered important in discussions of arthropod evolution.

The femoro-patellar joints of all the walking legs of *Limulus* (*Limulus polyphemus*) are hinge joints lacking extensor muscles (see Fig. 1). Nevertheless, in the power strokes of normal walking the femoro-patellar joints of *Limulus* perform up to 120 degrees of extension from the flexed state. This is the greatest angular displacement occurring at any joint in the legs. The mechanism of extension of these joints is not known. These extensions are important in performing walking motions of the legs, which propel the animal over intertidal mud flats and through surface sediments of the ocean floor.

In this paper I present data concerning femoro-patellar extension in *Limulus*. I propose and provide evidence for a mechanical scheme of extension of these extensorless hinge joints. I conclude that the mechanism is neither hydraulic nor elastic.

EXPERIMENTAL ANIMALS

Live specimens of *Limulus* were obtained commercially from Panacea, Florida, and collected at Beaufort, North Carolina. The animals used for pressure measurements ranged from 20.0 to 23.5 cm in length from the front of the carapace to the base of the telson. Larger or smaller animals were used for other work. Some animals arrived in poor condition, showed below normal activity and died a few days later. These animals also showed below normal blood pressures that were as low as zero in some cases; data from such animals are not included here. In one *Limulus* the blood pressure was very low but the animal appeared otherwise normal; these data are therefore included. All animals included became quite active when picked up and they lived in the laboratory for three or more weeks

even after various operations. The animals were kept in tanks containing aerated artificial sea water ("Instant Ocean," Aquarium Systems Inc., Wickliffe, Ohio) at 13° C.

NORMAL HINGE JOINT ANGLES

The maximum *in vivo* change in angle from the flexed position of the femoro-patellar joint ranged from 35 to 71 degrees provided the limb was not pushing against any object. This range of extension was determined from measurements (using a protractor) on each of several legs of six animals in both inverted and

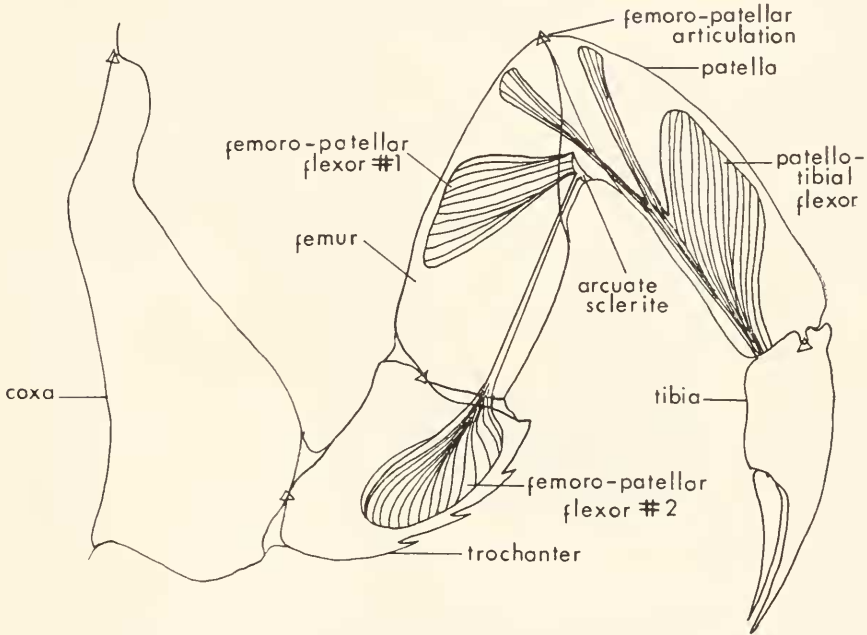


FIGURE 1. Muscles at the femoro-patellar joint of left leg V of *Limulus*. Strong flexors are attached to the arcuate sclerite in the ventral membrane of the joint. The two slender muscles attached near the joint are part of the patello-tibial system. Contraction elicited by electrical stimulation of each muscle near the femoro-patellar joint failed to extend this joint. Morphology of all the structures shown here is very similar in all the legs. Points of articulation are marked with Δ .

upright positions. When the limb had a purchase distally, as on the ground in walking, extension of up to 120 degrees from the flexed position was observed. Some experiments were done with the limb tips off the ground and some with the limb tips on the ground. In all cases the angles obtained at the femoro-patellar joint were compared only to the angles of this joint in the intact animal in the same position.

DENERVATION

Methods

If extension of the femoro-patellar joint were done by muscles, then denervation of all the leg muscles should eliminate the extension. The main leg nerve (the

only nerve entering the leg) was cut at the base of the coxa in intact animals with as little damage to the rest of the tissues as possible. In other animals the nerves were cut at other sites to localize the elements that performed the extension, as follows. Starting distally the nerve was cut at every joint in the leg. After each cut the minimum and maximum extensions of the femoro-patellar joint were determined. The maximum and minimum angles were previously measured in the intact animal. In every case the same operation was performed on the contralateral leg without cutting the nerve; this operation acted as a control for factors such as injury or loss of blood that might impair extension or activity in general.

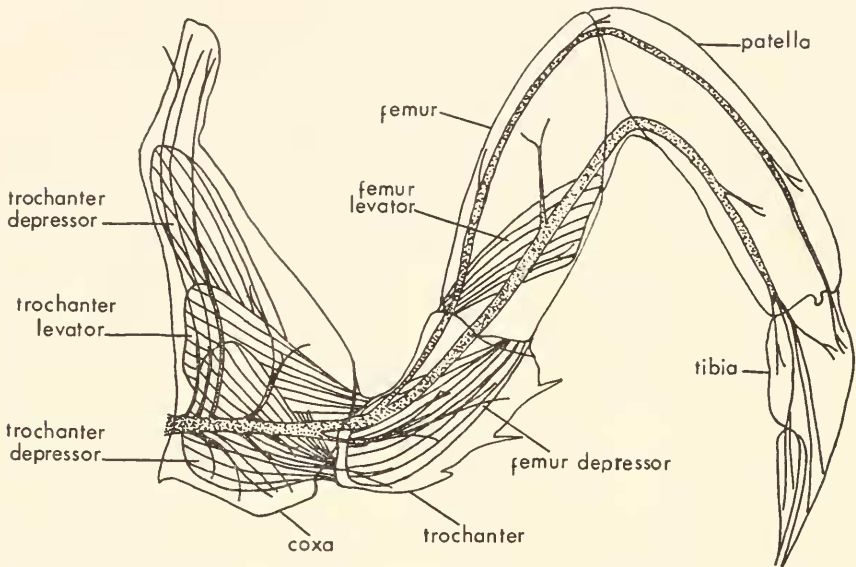


FIGURE 2. Nerves in left leg V of *Limulus*, anterior view. All structures shown here have very similar analogs in all walking legs. The nerves are shown in heavy stippling (detail added from Patten and Redenbaugh, 1899). The muscles shown are only those found in the coxa and trochanter (femoro-patellar flexor #2, which inserts in the trochanter, has been excluded: see Fig. 1). For musculature of the femur and patella, see Figure 1.

In the segments where the small leg nerve is also present (the main leg nerve splits into two branches in the trochanter; see Fig. 2), both nerves were cut to eliminate all innervation of the muscles in that segment.

Results

When all leg musculature was denervated by cutting the nerve at the base of the coxa no measurable extension of the femoro-patellar joint occurred. This indicated that some muscles were involved in the extension of this hinge joint. Since the above denervations were the only ones that completely eliminated extension of the femoro-patellar joint, muscles in the coxa must be involved in the extension of the hinge joint. When the nerve was cut at the coxa-trochanter joint (see Fig. 2), zero to 96% of the maximum *in vivo* extension was observed, indicating that the

muscles in the trochanter may also have a role in the extension. Denervation at sites other than the prosoma-coxa and coxa-trochanter joints in no way affected the extension of the femoro-patellar joint. A flexor of the femoro-patellar joint also attaches in the trochanter; however, cutting this muscle or the nerves that innervate it did not decrease the extension of the femoro-patellar joint.

MUSCLE STIMULATION

Methods

To identify possible muscular effectors of extension various muscles and muscle groups were stimulated electrically in excised legs, simultaneously measuring changes in femoro-patellar joint angle. The excised legs were placed in a position similar to the normal flexed position in the live *Limulus*. "Windows" were cut in the exoskeleton of each segment of the leg, leaving the muscles (except for the fasciae attached directly to that portion of the exoskeleton), joints and other structures intact. The muscles were then stimulated through the windows. Stimulation was done with silver wire electrodes and a square wave or induction stimulator at 20 volts. This experiment was done with the limb tip free and with the limb tip fixed (allowing movement at the proximal end in the case of the limb tip fixed).

Results

Normal extension of the femoro-patellar joint, both for the limb tip free and the limb tip held fixed, was produced only by contraction of the depressors of the trochanter or the depressors of the femur or both (see Fig. 2). The contraction of no other muscle produced extension of that hinge joint under these conditions.

OBSERVATIONS CONCERNING ELASTICITY

Methods

Excised legs were examined for a possible elastic source of hinge joint extension as follows. Excised legs were flexed manually to various angles and released after each forced flexure. Any changes in femoro-patellar joint angles following the flexures were recorded. This experiment was done on freshly excised legs with the muscles intact and was repeated after cutting the flexors of the femoro-patellar joint. The same tests were done for various orientations of the leg with respect to gravity.

The dorsal membrane of the hinge joint was pulled at either end to determine any changes in length, as measured by a millimeter ruler, resulting from tensile stress.

Results

No recoil, that is, no change in hinge joint angle, occurred after forced flexure of the joint in any case within the range of angles observed *in vivo*. There was no change in length measurable by the method used in the dorsal membrane of the hinge joint. No other structures were found which seemed in any way capable of elastically extending the femoro-patellar joint.

PRESSURE MEASUREMENTS

Methods

Measurements of pressure were made with a pressure transducer connected to a catheter fitted with an 18-gauge hypodermic needle at the other end. The transducer was a Sanborn model 267B physiological pressure transducer; it was coupled to a Beckman Offner type RS Dynograph amplifier-recorder. The transducer and catheter were filled with saline for fluid-system pressure measurements. The readings were taken with the needle inserted into the haemocoel through an arthroal membrane or a hole drilled in the exoskeleton. Possible interference by clotting was avoided by using a hypodermic needle sleeve which was cleared with a metal core before inserting the needle. After each measurement the transducer was checked with a known pressure and compared to the initial calibration; if the results of the two calibrations were not identical, the data from that measurement were discarded.

Measurements on live specimens of *Limulus* were made at various sites in the legs, including under the membrane of the femoro-patellar and tibio-tarsal joints and under the exoskeleton of the femur and of the patella. Blood pressure was also measured dorsally through the carapace in the region of the heart, and ventrally through the membranes of the prosoma (cephalothorax).

Results

The average prosomal pressure at rest for eight specimens of *Limulus* was 14.1 mm Hg \pm 11.6 (mean \pm standard deviation). The average prosomal pressure during activity for four specimens of *Limulus* was 23.8 mm Hg \pm 16.5. The comparison between these overall means does not show a statistically significant difference ($t = 1.23$, $0.20 > p > 0.10$). However, the difference between resting and active mean pressures is statistically significant in every case if the comparison is made for each animal ($t = 2.93$, $0.01 > p > 0.005$ in one case, and the means were separated by more than two standard deviations in all other cases).

The activities included in the active measurements were struggling by the legs, flapping of the gills, and flexion and extension of the prosoma-opisthosomal joint.

Most measurements were made ventrally through the membranes of the prosoma; four measurements were made dorsally in the region of the heart. The latter values were all within the range of the pressures measured ventrally in the same animal.

The *in vivo* pressure in the legs did not change significantly with extension of the femoro-patellar joint. For seven specimens of *Limulus* the mean pressure in the legs while the femoro-patellar joint was fully flexed was 5.1 mm Hg \pm 1.8; while the same joint was extended the mean pressure was 6.2 mm Hg \pm 2.4. When these means are compared the difference is not statistically significant ($t = 0.97$, $0.15 > p > 0.10$). Comparisons of pressures in flexed and extended legs for individual animals show statistically significant differences in only three out of eight cases. Pressures recorded while the femoro-patellar joint was in intermediate positions were between the flexed and extended values.

DETERMINATION OF PRESSURE REQUIRED FOR EXTENSION

Methods

The pressure required to extend hydraulically the femoro-patellar joint of a detached leg was determined by the following procedure. Legs were excised from nine live specimens of *Limulus* at the base of the trochanter; the leg haemocoel was then closed off with a rubber stopper (see Fig. 3) and the internal pressure was then increased by injecting saline with a syringe into the closed system. Simultaneously, the internal pressure in the leg was monitored and the change in femoro-patellar joint angle was measured with a protractor. Measurements of the pressure through the membrane of the hinge joint and through the exoskeleton gave the same results

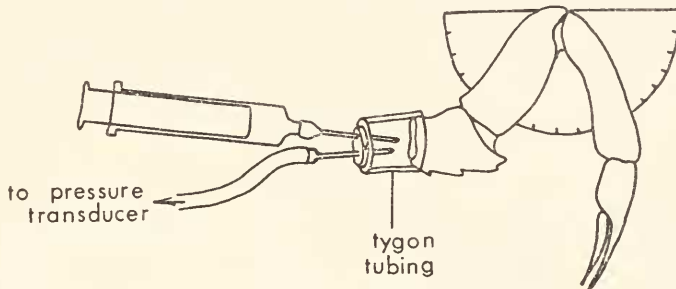


FIGURE 3. Determination of pressure required for extension.

as measurements through the stopper. Subsequent measurements were therefore made through the stopper, which was more convenient.

Results

The pressures measured *in vivo* in the legs are much smaller than the pressures required to extend the hinge joint hydraulically. Figure 4 shows the pressure required for extension of the femoro-patellar joint determined for nine animals. The pressure needed to produce the observed extension of about 50 degrees in this position was about 150 mm Hg according to this graph. Also on Figure 4 are the mean *in vivo* leg blood pressures. These means are the normal blood pressures described above, measured *in vivo* in the leg while the femoro-patellar joint is fully flexed and while it is fully extended. The mean leg blood pressures are only enough to account for about a three-degree change in joint angle. In no case did the *in vivo* leg pressures reach a value that could account for a ten-degree extension as determined by the graph, whereas normal extensions by the animal ranged from 35 to 71 degrees under these conditions.

Cutting all the flexors reduced the pressure needed for full extension of the hinge joint by only 8%. Thus any overestimation of the pressure needed for extension due to overcoming the friction or the force of contraction of the flexors amounted to at most 8%. In fact, the error from this source was probably much smaller since in the living animals the force of extension must overcome some resistance in the flexors even when these muscles are relaxed.

DISCUSSION

Indirect muscular mechanism

The results of denervation and muscle stimulation and the observation of walking movements in live specimens of *Limulus* support the hypothesis that extension of the femoro-patellar joint is performed by a simple mechanical system, the effectors of which are the depressors of the trochanter, the depressors of the femur, and the coxal remotors (term of Manton, 1964). With this system, all observed extension movements of the extensorless femoro-patellar joints of *Limulus* can be explained. The various cases of extension observed in the intact animal are described below; following each is an explanation of the movement in terms of the proposed mechanical scheme.

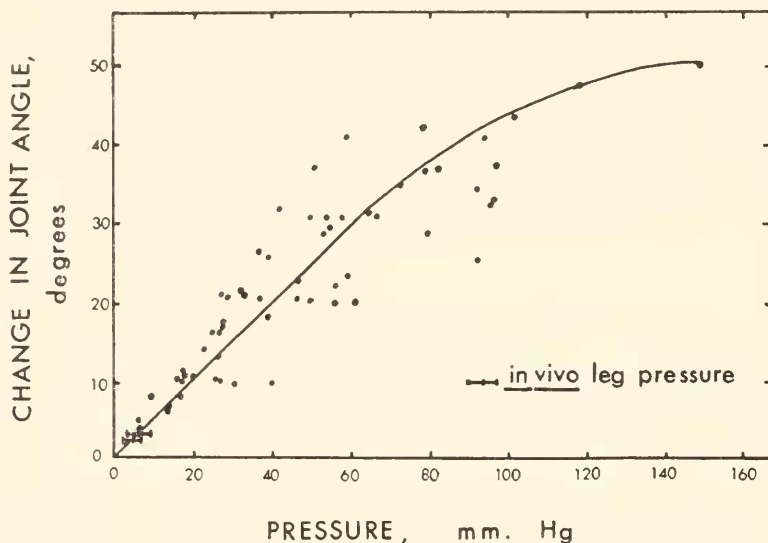


FIGURE 4. Extension of the femoro-patellar joint with artificial increase in internal hydrostatic pressure. The *in vivo* leg pressures are also shown (mean and one standard deviation on either side of the mean) to show contrast between the pressures required to extend the leg and the pressure actually found in the leg.

The first four pairs of walking legs (prosomal appendages II through V) may perform up to 70 degrees of extension of the femoro-patellar joint when off the ground. When the animal is upside-down in air, the femoro-patellar joint usually extends about 50 degrees as the limbs are pushed upwards, until a point is reached when the patella and more distal segments fall inwards to the normal flexed position. These movements can be effected by the action of the depressors of the trochanter and of the femur, which push the leg upwards in this position, and by the weight of the patella and more distal segments, which causes the femoro-patellar joint to extend (see Fig. 5a). Once the patella and more distal segments reach the point where their weight tends to pull them inwards (Fig. 5b), they fall passively over into the flexed position. These extension movements and those described below require previous relaxation of the flexors of the femoro-patellar joint.

In the normal upright position, the first four pairs of walking legs also may extend when the legs are off the ground. In the recovery strokes of walking, the femoro-patellar joint extends about 30 degrees, at which point the patella and more distal segments are in a vertical position. No further extension occurs in the forward or recovery strokes of walking. These movements may be performed by the depressors of the trochanter and femur, again, bringing the entire leg downwards (see Fig. 6a): after relaxation of the flexors of the femoro-patellar joint,

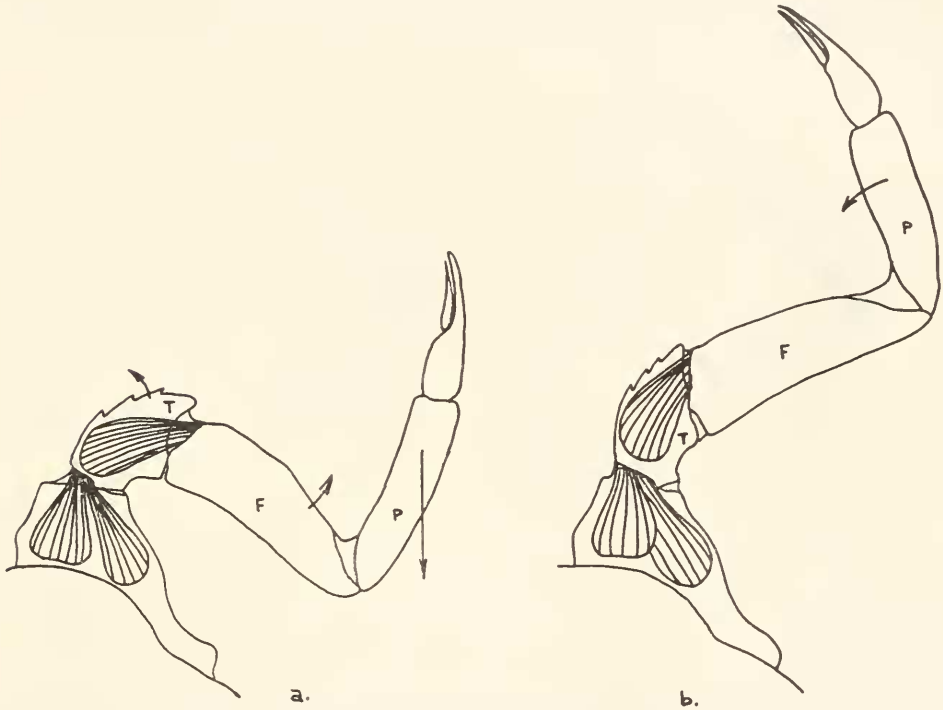


FIGURE 5. Extension of right leg V (anterior view) when the animal is upside-down. In this diagram the only muscles shown are those thought to extend the femoro-patellar joint. a: leg beginning extension. b: leg extended, patella and more distal segments about to fall into the flexed position. T = trochanter, F = femur, P = patella.

the depression of the basal segments of the limb and the weight of the patella and more distal segments would extend the femoro-patellar joint (see Fig. 6b).

When the limbs are on the ground, the same movements are observed and the same explanations may be applied, with a few additions. When legs II through V have a purchase distally they can perform additional extension of the femoro-patellar joint (up to 120 degrees). As the depressors push the leg down, the resistance of the ground at the limb tip causes forward or upward movement of the body and is accompanied by extension of the femoro-patellar joint. Also, the depressors of the tibia must be contracting to keep the patello-tibial joint rigid as the leg bears down on the ground and the hinge joint extends. The same "postural"

contractions must occur at most hinge joints whose muscles are not direct effectors of the movements being performed in walking.

The last pair of legs (prosomal appendages VI) has so far been excluded from the above descriptions because of its special movements. Even when these legs are off the ground, they may perform up to 120 degrees of extension of the femoro-patellar joints, provided the legs are positioned almost horizontally (see Fig. 7). The legs are kicked backwards, resulting in a straight-line configuration of all the segments in the legs. This results in a nearly 180-degree angle at the extensorless femoro-patellar joint, or a total extension of 120 degrees from the flexed position for most sixth legs. The same movement occurs when the animal is upside-down.

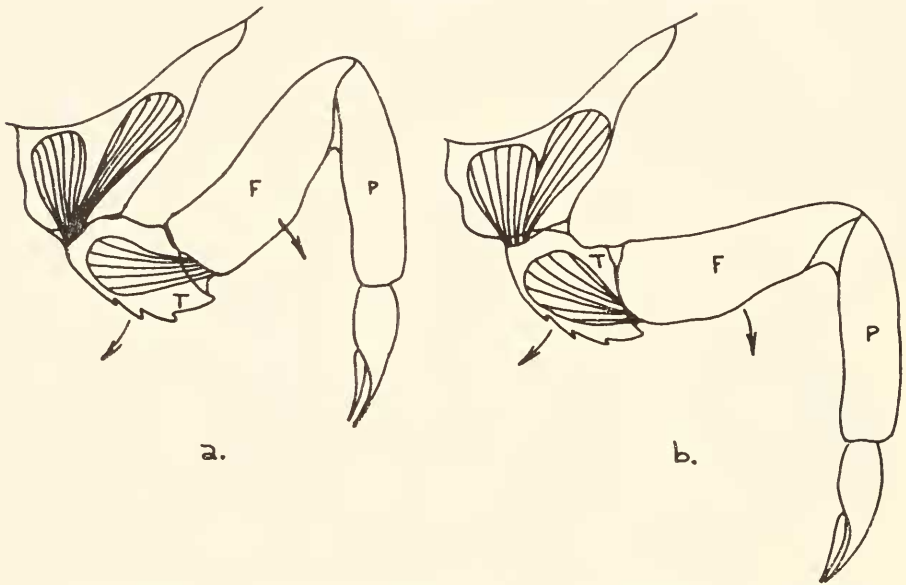


FIGURE 6. Extension of left leg V (anterior view) when the animal is in the normal upright position. The only muscles shown are those thought to effect the extension of the hinge joint. a: leg elevated. b: leg depressed and extended. T = trochanter, F = femur, P = patella.

The backwards rotation of the coxa is doubtless due to the coxal remotor muscles. Since the leg is now in a horizontal position, the axes of the joints are in a vertical position; therefore the weight of the segments beyond the femoro-patellar joint does not tend to flex that joint. The other pairs of legs also have coxal remotor muscles; however, the motions just described are not performed to any significant extent because rotation of the coxa is limited by the closely following coxae of more posterior legs. Note the position of the coxa of leg V in Figure 7b(ii).

The last pair of legs are the legs which seem to do most of the work in propelling the animal through mud and sand by rapid straightenings and application of the large area of their distal flattened spines to the substrate. The locomotion of *Limulus* through mud or on dry ground can be observed to proceed in two phases. There is a period of motion, then a pause, and the cycle repeats itself. This pattern

is due in the movement phase to the power stroke of the rear legs and in the pausing phase to the recovery stroke of the rear legs. The other legs also perform stroking movements in walking, but these strokes do not always coincide with phases of movement of the animal. Since the tips of these legs are chelae instead of flattened plates, they can get little purchase in mud or sand unless there are configurations in the substratum which can be grasped by a chela. Thus most of the forward locomotion in *Limulus* is effected with the last pair of legs.

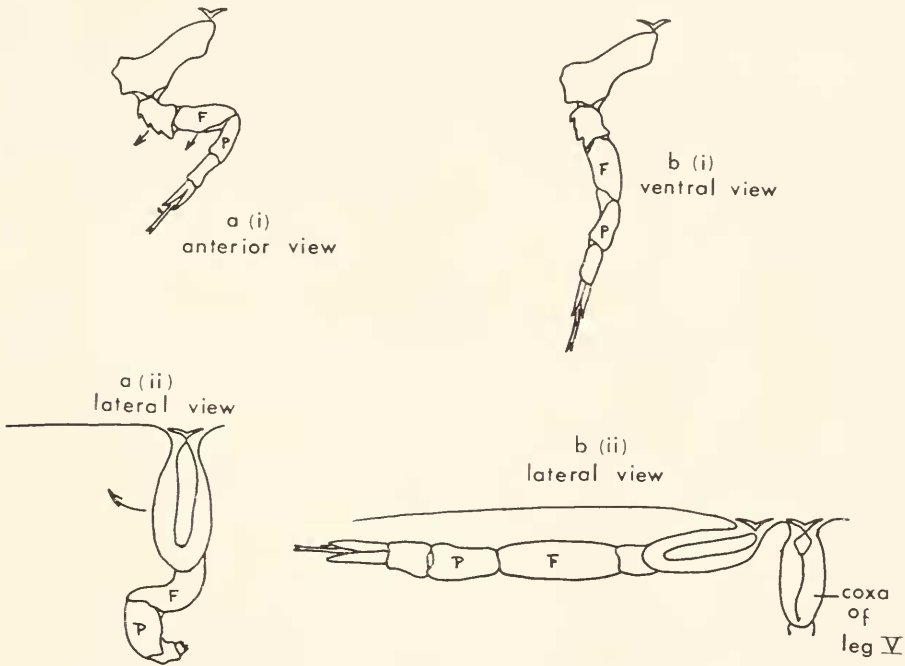


FIGURE 7. Extension of left leg VI. a: leg in normal flexed position. b: leg fully extended. The anterior view shown in a(i) is before rotation of the coxa; once rotated, as in b(i), the same surface becomes ventral. F = femur, P = patella.

The extension movements of the last pair of legs, when their tips are on the ground, are basically those observed in the same legs when their tips are off the ground; that is, the coxa rotates posteriorly, the legs are kicked back, and the hinge joint extends 120 degrees as the body moves forward. Thus the same mechanism may be applied to explain these extension movements.

The schemes that Manton (1958a, 1958b) outlined for myriapods, scorpions, and spiders are similar to that presented here for *Limulus*. However, in myriapods and scorpions rotation of the hinge joint surface occurs during extension of those joints in all the legs. These animals show varied and intricate morphological specializations for leg rotation (Manton, 1958a, 1958b). In *Limulus*, only the last pair of legs performs considerable rotation; and the weight of the leg is an important effector of extension of the hinge joints of all the legs.

Elasticity

The evidence against an elastic mechanism of femoro-patellar extension comes from several sources. In an excised leg no recoil occurs after a forced flexion as reported in the results, whether or not the muscles are cut. Thus neither the muscles nor the dorsal hinge joint membrane seem to provide energy for extension. Also, the dorsal hinge joint membrane does not stretch visibly when pulled; thus there is no evidence that it is a storage site for elastic force causing extension.

Snodgrass (1952) proposed that in the case of the prosoma-coxal joints of *Limulus*, elasticity, either of the muscles or the membranes, may have a role in extension, but there is no evidence available to explain or support its existence. Manton (1964) proposed that there are extensors in those joints of *Limulus*. The muscles she describes as extensors are the two attaching on either side of and nearest to the pleurocoxal articulation. In any case, these hinge joints perform little extension even during feeding movements, which involve motions of the gnathobases of the coxae.

Hydraulic mechanism

Manton (1958a) proposed, as a general rule for arthropods, that extension is performed hydraulically when the limb tip is off the ground but that a hydraulic mechanism could not account for the power strokes when the limb tip is on the ground. Parry and Brown, however, have made measurements of pressure which indicate that in spiders a hydraulic mechanism can account not only for the power strokes of walking (1959a) but also for the jump of salticid spiders (1959b). Because of the marked similarity in morphology between the hinge joints of *Limulus* and those of spiders, it has been assumed that the horseshoe crab also has a hydraulic mechanism of leg extension (see Pringle, 1956).

The average prosomal blood pressures of 14.1 mm Hg at rest to 23.8 mm Hg during activity found in *Limulus* are similar to the "typical" blood pressures found in most arthropods (Prosser and Brown, 1961; Inada, 1947). These pressures are much lower than the blood pressure of spiders with hydraulic leg extension. The blood pressure of *Tegenaria* ranges from 50 mm Hg at rest to about 400 mm Hg with activity (Parry and Brown, 1958a).

As seen in the results the average leg blood pressure in *Limulus* is 5.1 mm Hg for the flexed position of the femoro-patellar joint and 6.2 mm Hg for the extended position of the same joint; as mentioned before, this difference is not statistically significant. If a hydraulic mechanism of hinge joint extension were operating, a large and reliable difference in blood pressure would be expected when the extension occurred; namely, a difference in pressure which would be great enough to account for extension of the hinge joint. Not only is there no significant change in *in vivo* blood pressure in the leg on flexion and extension of the hinge joint, but, as can be seen in Figure 4, the blood pressure measured in the legs is far below the pressure needed to account for the amount of extension that occurs at the femoro-patellar joint in the living animal.

In spiders, bleeding or dehydration leads to loss of the ability to extend the hinge joints (Ellis, 1944). Further evidence supporting the conclusion that the extension of the hinge joints of *Limulus* is not effected hydraulically is the fact that

all normal leg movements, including the maximum normal extension of the femoro-patellar joint, are observed in *Limulus* after massive blood loss. Although this observation still leaves open the possibility that in *Limulus* a local change in blood pressure could effect the extension, this seems unlikely since (1) pressure measurements in the legs were made near or at the hinge joint and (2) no structure or structures were found in the legs or near the legs that could isolate or pressurize a section of the leg or other compartment. Further, if a hole is drilled in the exoskeleton adjacent to the hinge joint at various sites, there is no impairment of extensor activity.

Haemocoelic hydraulic locomotory mechanisms probably evolved as an adaptation for burrowing in soft-bodied animals (Manton, 1961). However, with the acquisition of a stiff integument, most arthropods use direct muscular locomotory mechanisms rather than hydraulic systems. Notable exceptions occur in some or all spiders and perhaps in barnacles (Cannon, 1947) and some myriapods (Manton, 1958a), although direct evidence is lacking. In spite of the morphological similarity between the legs of *Limulus* and those of spiders, *Limulus*, according to the results of this study, has no hydraulic means of leg extension. At least partial hydraulic extension, however, occurs in spiders and may occur in other terrestrial arthropods with several hinge joints in the legs. Without hydraulic extension such animals might have difficulties in supporting their body weights, as happens in spiders following bleeding or dehydration (Ellis, 1944).

I wish to thank Dr. S. A. Wainwright for his advice throughout the course of this work and my husband for his extensive help. I was supported in part by a grant from the Cocos Foundation.

SUMMARY

The femoro-patellar hinge joints of *Limulus* show considerable extension in spite of their lack of extensor muscles.

I propose a mechanical scheme for extension of these extensorless hinge joints. The extension is performed by the combined action of the depressors of the trochanter, the depressors of the femur, and, especially in the case of the powerful last pair of legs, the coxal remotor muscles. The femoro-patellar joint extends passively due to (1) the effect of these muscles, (2) the force of the weight of the leg when off the ground, and (3) the force exerted by the limb tip against the substratum when the leg is on the ground.

Measurements of hydrostatic pressure in *Limulus* indicate that a hydraulic mechanism is not an effector of femoro-patellar extension. There is no evidence that an elastic mechanism functions in extending this joint.

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